

CLAIMS

1. A substantially purified or isolated nucleic acid or nucleic acid fragment encoding an organic acid biosynthesis enzyme polypeptide selected from the group consisting of a citrate synthase (CS) polypeptide; a CS-like polypeptide;
5 a malate dehydrogenase (MDH) polypeptide; a MDH-like polypeptide; a phosphoenolpyruvate carboxylase (PEPC) polypeptide; and a PEPC-like polypeptide; or a functionally active fragment or variant of such a polypeptide, from a clover (*Trifolium*), medic (*Medicago*), ryegrass (*Lolium*) or fescue (*Festuca*) species.
- 10 2. A nucleic acid or nucleic acid fragment wherein said nucleic acid or nucleic acid fragment is from white clover (*Trifolium repens*) or perennial ryegrass (*Lolium perenne*).
3. A nucleic acid or nucleic acid fragment encoding a CS or CS-like polypeptide and including a nucleotide sequence selected from the group
15 consisting of (a) sequences shown in Figures 1, 3, 4, 6, 7, 9, 99, 101, 102, 104, 114, 118 and 122 hereto (SEQ ID NOS 1, 3 to 10, 11, 13 to 16, 17, 19, 327, 329 to 335, 336, 338 to 344, 349, 351, 353 respectively); (b) complements of the sequences recited in (a); (c) sequences antisense to the sequences recited in (a) and (b); (d) functionally active fragments and variants of the sequences recited in
20 (a), (b) and (c); and (e) RNA sequences corresponding to the sequences recited in (a), (b), (c) and (d).
4. A nucleic acid or nucleic acid fragment encoding a MDH or MDH-like polypeptide and including a nucleotide sequence selected from the group
25 consisting of (a) sequence shown in Figures 11, 13, 14, 16, 17, 19, 21, 23, 25, 26, 28, 30, 31, 33, 35, 37, 38, 40, 55, 57, 58, 60, 61, 63, 64, 66, 67, 69, 70, 72, 73, 75, 76, 78, 79, 81, 82 and 84 hereto (SEQ ID NOS 21, 23 to 29; 30, 32 to 33, 34, 36, 38, 40, 42 to 43, 44, 46, 48 to 110, 111, 113, 115, 117 to 182, 183, 185, 205, 207 to 217, 218, 220 to 251, 252, 254 to 270, 271, 273 to 275, 276, 278 to 287, 288, 290 to 292, 293, 295 to 296, 297, 299 to 301, 304 to 305, 306, 308); (b)
30 complements of the sequences recited in (a); (c) sequences antisense to the sequences recited in (a) and (b); (d) functionally active fragments and variants of the sequences recited in (a), (b) and (c); and (e) RNA sequences corresponding to the sequences recited in (a), (b), (c) and (d).

5. A nucleic acid or nucleic acid fragment encoding a PEPC or PEPC-like polypeptide and including a nucleotide sequence selected from the group consisting of (a) sequences shown in Figures 42, 44, 46, 47, 49, 51, 53, 86, 88, 89, 91, 92, 94, 95, 97 and 110 hereto (SEQ ID NOS 187, 189, 191 to 197, 199, 201, 203, 310, 312 to 314, 315, 317 to 318, 319, 321 to 322, 323, 325 and 347 respectively); (b) complements of the sequences recited in (a); (c) sequences antisense to the sequences recited in (a) and (b); (d) functionally active fragments and variants of the sequences recited in (a), (b) and (c); and (e) RNA sequences corresponding to the sequences recited in (a), (b), (c) and (d).

6. A construct including one or more nucleic acids or nucleic acid fragments according to any one of claims 1 to 5.

7. A construct according to claim 6 including nucleic acids or nucleic acid fragments encoding both (a) a CS polypeptide or a CS-like polypeptide and (b) a MDH polypeptide or a MDH-like polypeptide.

8. A construct according to claim 6 including nucleic acids or nucleic acid fragments encoding both (a) a CS polypeptide or a CS-like polypeptide and (b) a PEPC polypeptide or a PEPC-like polypeptide.

9. A construct according to claim 6 including nucleic acids or nucleic acid fragments encoding both (a) a MDH polypeptide or a MDH-like polypeptide and (b) a PEPC polypeptide or a PEPC-like polypeptide.

10. A construct according to claim 6 including nucleic acids or nucleic acid fragments encoding all three of (a) a CS polypeptide or a CS-like polypeptide; (b) a MDH polypeptide or a MDH-like polypeptide; and (c) a PEPC polypeptide or a PEPC-like polypeptide.

11. A construct according to any one of claims 6 to 10 wherein the one or more nucleic acids or nucleic acid fragments are operably linked to one or more regulatory elements, such that the one or more nucleic acids or nucleic acid fragments are each expressed.

12. A construct according to Claim 11, wherein the one or more regulatory elements include a promoter and a terminator, said promoter, nucleic acid or nucleic acid fragment and terminator being operably linked.

13. A plant cell, plant, plant seed or other plant part, including a construct according to any one of claims 6 to 12.

14. A plant, plant seed or other plant part derived from a plant cell or plant according to Claim 13.

5 15. A method of modifying one or more of organic acid synthesis; organic acid secretion; nutrient acquisition; aluminium and acid soil tolerance; or nitrogen fixation and nodule function; in a plant, said method including introducing into said plant an effective amount of a nucleic acid or nucleic acid fragment according to any one of claims 1 to 5, or a construct according to any one of
10 claims 6 to 12.

16 A method according to claim 15 wherein said method includes introducing into said plant effective amounts of nucleic acids or nucleic acid fragments encoding both (a) a CS polypeptide or CS-like polypeptide and (b) a MDH polypeptide or MDH-like polypeptide.

15 17. A method according to claim 15 wherein said method includes introducing into said plant effective amounts of nucleic acids or nucleic acid fragments encoding both (a) a CS polypeptide or a CS-like polypeptide and (b) a PEPC polypeptide or a PEPC-like polypeptide

18. A method according to claim 15 wherein said method includes
20 introducing into said plant effective amounts of nucleic acids or nucleic acid fragments encoding both (a) a MDH polypeptide or a MDH-like polypeptide and (b) a PEPC polypeptide or a PEPC-like polypeptide.

19. A method according to claim 15 wherein said method includes introducing into said plant effective amounts of nucleic acids or nucleic acid
25 fragments encoding all three of (a) a CS polypeptide or a CS-like polypeptide; (b) a MDH polypeptide or a MDH-like polypeptide; and (c) a PEPC polypeptide or a PEPC-like polypeptide.

20. A method according to any one of claims 15 to 19 wherein the method is modifying nutrient acquisition and the nutrient is phosphorous.

21. Use of a nucleic acid or nucleic acid fragment according to any one of claims 1 to 5, and/or nucleotide sequence information thereof, and/or single nucleotide polymorphisms thereof as a molecular genetic marker.

22. A substantially purified or isolated nucleic acid or nucleic acid
5 fragment including a single nucleotide polymorphism (SNP) from a nucleic acid fragment according to any one of claims 1 to 5.

23. A nucleic acid or nucleic acid fragment including an SNP according to Claim 22, wherein said nucleic acid or nucleic acid fragment is from white clover (*Trifolium repens*) or perennial ryegrass (*Lolium perenne*).

10 24. A substantially purified or isolated polypeptide from a clover (*Trifolium*), medic (*Medicago*), ryegrass (*Lolium*) or fescue (*Festuca*) species, selected from the group consisting of CS and CS-like, MDH and MDH-like and PEPC and PEPC-like; and functionally active fragments and variants thereof.

25. A polypeptide according to Claim 24, wherein said polypeptide is
15 from white clover (*Trifolium repens*) or perennial ryegrass (*Lolium perenne*).

26. A polypeptide encoded by a nucleic acid or nucleic acid fragment according to any one of claims 1 to 5.

27. A polypeptide according to Claim 24 or 25, wherein said polypeptide is CS or CS-like and includes an amino acid sequence selected from the group
20 consisting of sequences shown in Figures 2, 5, 8, 10, 100, 103, 115, 119, 123 hereto (SEQ ID NOS 2, 12, 18, 20, 328, 337, 350, 352 and 354 respectively); and functionally active fragments and variants thereof.

28. A polypeptide according to Claim 24 or 25, wherein said polypeptide is MDH or MDH-like and includes an amino acid sequence selected from the
25 group consisting of sequences shown in Figures 12, 15, 18, 20, 22, 24, 27, 29, 32, 34, 36, 39, 41, 56, 59, 62, 65, 68, 71, 74, 77, 80, 83 and 85 hereto (SEQ ID NOS 22, 31, 35, 37, 39, 41, 45, 47, 112, 114, 116, 184, 186, 206, 219, 253, 272, 277, 289, 294, 297, 303, 307 and 309, respectively) and functionally active fragments and variants thereof.

30 29. A polypeptide according to Claim 24 or 25, wherein said polypeptide is PEPC or PEPC-like and includes an amino acid sequence selected from the

group consisting of sequences shown in Figures 43, 45, 48, 50, 52, 54, 87, 90, 93, 96, 98 and 111 hereto (SEQ ID NOS 188, 190, 198, 200, 202, 204, 311, 316, 320, 324, 326, and 348, respectively); and functionally active fragments and variants thereof.